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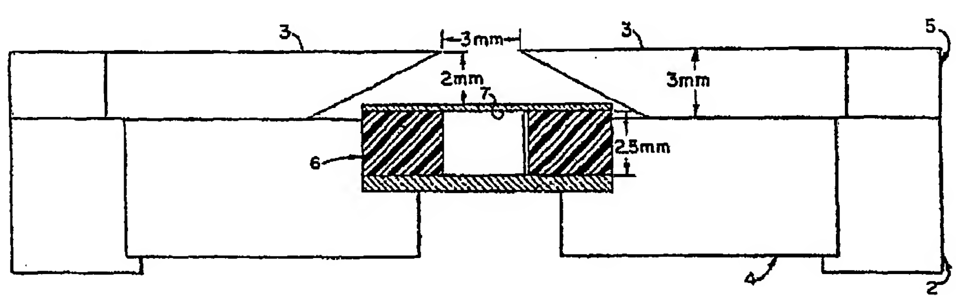
(54) Title: MAGNETIC SEPARATION APPARATUS AND METHODS



(57) Abstract: Apparatuses and methods for separating, immobilizing, and quantifying biological substances from within a fluid medium. Biological substances are observed by employing a vessel (6) having a chamber therein, the vessel comprising a transparent collection wall (5). A high internal gradient magnetic capture structure may be on the transparent collection wall (5), magnets (3) create an externally-applied force for transporting magnetically responsive material toward the transparent collection wall (5). V-shaped grooves on the inner surface of the viewing face of the chamber provide uniform . The invention is also useful in conducting quantitative analysis and sample preparation in conjunction with automated cell enumeration techniques.

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MAGNETIC SEPARATION APPARATUS AND METHODS

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Cross-Reference to Related Applications

This is a continuation-in-part of 10/733829, filed on December 10, 2003, now allowed, which is a division of U.S. 6,790,366, issued on September 14, 2004, which is a 371 of PCT/US99/28231, filed on November 30, 1999, which is a continuation-in-part of U.S. Application No. 09/201,603, filed November 30, 1998, now U.S. Patent No. 6,136,182 which is a continuation-in-part of U.S. Application No. 08/867,009, filed June 2, 1997, now U.S. Patent No. 5,985,153, which claims the benefit of U.S. Provisional Application No. 60/019,282, filed June 7, 1996, and claims the benefit of U.S. Provisional Application No. 60/030,436, filed November 5, 1996. Application No, 09/856,672, now allowed, U.S. Patent No. 6,136,182 and U.S. Patent No. 5,985,153 are all incorporated in full by reference herein.

BACKGROUND

The present invention relates to improved apparatus and methods for performing qualitative and quantitative analysis of microscopic biological specimens. In particular, the invention relates to such apparatus and methods for isolating, collecting, immobilizing, and/or analyzing microscopic biological specimens or substances which are susceptible to immunospecific or non-specific binding with magnetic-responsive particles having a binding agent for producing magnetically-labeled species within a fluid medium. As used herein, terms such as "magnetically-labeled specimen" shall refer to such biological specimens or substances of investigational interest which are susceptible to such magnetic labeling.

U.S. Patent No. 5,985,153 describes an apparatus and method wherein an external magnetic gradient is employed to attract magnetically labeled target specimens present in a collection chamber to one of its surfaces, and where an internal magnetic gradient is employed to obtain precise alignment of those specimens on that surface. The movement of magnetically labeled biological specimens to the collection surface is obtained by applying a vertical magnetic gradient to move the magnetically labeled biological specimens to the collection surface. The collection surface is provided with a ferromagnetic capture structure, such as plurality of ferromagnetic lines supported on an optically transparent (viewing) face of a sample chamber.

Once the magnetically labeled biological specimens are pulled sufficiently close to the surface by the externally applied gradient, they come under the influence of an intense local gradient produced by the ferromagnetic collection structure and are immobilized at positions laterally adjacent thereto. The local gradient preferably exceeds adhesion forces which can hold the biological specimens to the transparent surface after they collide with the surface. Alternatively, the adhesiveness of the surface must be sufficiently weak to allow the horizontal magnetic force to move the magnetically labeled biological specimens towards the ferromagnetic structures. The smoothness and the hydrophobic or hydrophilic nature of the surface are factors that can influence the material chosen for the collection surface or the treatment of this surface to obtain a slippery surface.

U.S. 10/733829 and U.S. 6,790,366 describe methods and apparatus for separating, immobilizing, and quantifying biological substances in a fluid sample, incorporating the principles of the externally applied gradient described above, and further incorporate a high internal gradient magnetic capture structure on the transparent collection wall. The capture structure encourages a uniform alignment of caputred biological substances for quantitative analysis with automated enumeration techniques.

In accordance with the present invention, there are described further alternative embodiments and improvements for the collection chamber whereby the internal magnetic capture sturcture is used in conjunction with small V-shaped grooves on the fluid side of the optically transparent (viewing) face of the chamber to align the target specimens for automated optical analysis. A prefereed embodiment of the present invention replaces the internal magnetic capture structure with small V-shaped grooves on the fluid side of the optically transparent (viewing) face of the chamber, and with the optimum dilution of magnetically-labeled specimens provides an alignment surface for automated optical analysis. In both embodiments, magnetically-labeled specimens and unbound magnetic particles move toward the inner surface of the chamber's viewing face, under the influence of the externally applied magnetic gradient: When they approach the surface, they come in contact with the slope of the V-shaped groove, forcing the magnetically-labeled specimens and unbound magnetic particles to move to the top of the groove. At the top of the V-shaped groove is a small chimney-shaped component with a width of approximately 2 to 3 μm which stops the magneticall-labeled specimens and allows the unbound magnetic particles to move further up into the chimney structure and outside the focal plane, used in optical analysis. This allows for alignment of the cell population in a profile that allows easier scanning with minimization of nonhomogenously illuminated cell and provides an image of the cells without the interferring ferrofluid. In the

preferred embodiment, the need for internal magnetic capture structures, previously described, is not present, thus reducing the overall manufacturing cost of the viewing chamber.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A is a schematic diagram of a magnetic separator.

FIG. 1B shows the magnetic field provided in the magnetic separator of FIG. 1A.

FIGS. 2A-C are microphotographs of specimens collected in a magnetic separator.

FIGS. 3A and 3B are alignment lines induced by the extra magnetic field from Ni lines (A) or V-shaped grooves (B), both in the presence of the external magnetic field. Values D and L are the main parameters of the capture structure. L is the length of the flat horizontal area and D is the spacing of the grooves. The angle of 70.5° is described for the V-groove design shown, but it is understood that any angle design may be appropriate.

FIGS. 4A, 4B and 4C are successive schematic views showing a method of measuring particle density in a fluid having an unknown particle density.

FIG. 5 is a schmatic of the proces steps in BHF etching. First, a thin layer of SiO_2 (500 nm) is grown on the wafer by steam oxidation. A layer of photoresist is added and then selectively removed at the parts where further etching should occur. This is done with a lithography mask that contains the patterns to be etched. Then the BHF is introduced, removing the SiO_2 at places were there is no etch mask (photoresist). Finally, the layer of photoresist is removed and only the thin layer of SiO_2 is left.

FIG. 6 is a schematic illustration of PDMS molding.

FIG. 7 is the transmission spectrum of a PDMS slab approximately 1 mm thick. Typical transmission ranges are from 95% to 99% between 400 and 900 nm.

FIG. 8 shows a schematic illustration of V-grooves. L is the width of the horizontal area in the grooves and D is the spacing of the grooves. Cell alignment is shown with the arrow.

FIG. 9 shows a chimney-like design for removing the ferrofluid from the focal plane.

FIGS. 10A and 10B show the image of Hela cells in the V-grooves at several focal planes in DAPI and Cytokeratin-PE treated cells, respectively. Panel A shows several Hela cells aligned vertically for different points of focus within the cell. The numbers represent the values as obtained from imaging, indicating the point of focus in micrometers, using DAPI. Panel B shown the same with the phycoeurthrin (PE) labeled cytokeratin.

DETAILED DESCRIPTIONS

I. Vertical Gradient Collection and Observation of Target Specimens

Target specimens such as cells, cell debris, and cell components are collected against a collection surface of a vessel without subsequent alignment adjacent to a ferromagnetic collection structure. These cells include white blood cells, cells of epithelial origin, endothelial cells, fungal cells, and bacterial cells. The collection surface is oriented perpendicular to a magnetic field gradient produced by external magnets. In this embodiment, magnetic nanoparticles and magnetically labeled biological specimens are collected in a substantially homogeneous distribution on the optically transparent face of the chamber while non-selected entities remain below in the fluid medium. This result can be accomplished by placing a chamber in a gap between two magnets arranged as shown in FIG. 1A, such that the chamber's transparent collection surface is effectively perpendicular to a vertical field gradient generated by external magnets 3. The magnets 3 have a thickness of 3 mm, and are tapered toward a gap of 3 mm. The magnets 3 are held in a yoke 1, which rests atop a housing 2. A vessel support 4 holds the vessel 6 in a region between the magnets where the lines of magnetic force are directed substantially perpendicular to the collection surface 5 of the vessel 6. The collection surface of the vessel is preferably formed of a 0.1 mm thick polycarbonate member. The collection surface is parallel to, and 2 mm below, the upper surface of the external magnets 3. The space between the inner, top surface edges of the magnets is 3 mm.

The taper angle of the magnets 3 and the width of the gap between the two magnets determine the magnitude of the applied magnetic field gradient and the preferable position of the collection surface of the vessel. The field gradient produced by the magnets can be characterized as having a substantially uniform region, wherein the gradient field lines are substantially parallel, and fringing regions, wherein the gradient field lines diverge toward the magnets. FIG. 1B shows mathematically approximated magnetic field gradient lines for such a magnet arrangement. The magnetic field lines (not shown) are predominantly parallel to the chamber surface while the gradient lines are predominantly perpendicular to it. To collect a uniformly distributed layer of the target specimens, the vessel is positioned to place the chamber in the uniform region such that there are substantially no transverse magnetic gradient components which would cause lateral transport of the magnetically labeled biological specimens to the collection surface.

To illustrate the collection pattern of magnetic material on the collection surface area, a chamber with inner dimensions of 2.5 mm height (z), 3 mm width (x) and 30 mm length (y) was filled with 225 μl of a solution containing 150 nm diameter magnetic beads and placed in between the magnets as illustrated in FIG. 1A. The magnetic beads moved to the collection

surface and were distributed evenly. When the vessel was elevated relative to the magnets, such that a significant portion of the top of the vessel was positioned in a fringing region, significant quantities of the magnetic particles parallel toward and accumulated at respective lateral areas of the collection surface positioned nearest the magnets.

In order to enhance uniformity of collection on the collection surface, the surface material can be selected or otherwise treated to have an adhesive attraction for the collected species. In such an adhesive arrangement, horizontal drifting of the collected species due to any deviations in positioning the chamber of deviations from the desired perpendicular magnetic gradients in the "substantially uniform" region can be eliminated.

An example of the use of the present embodiment discussed device is a blood cancer test. Tumor derived epithelial cells can be detected in the peripheral blood. Although present at low densities, 1-1000 cells per 10 ml of blood, the cells can be retrieved and quantitatively analyzed from a sample of peripheral blood using an anti-epithelial cell specific ferrofluid. FIG. 2 illustrates an example of the use of the magnets and the chamber without the influence of a capture structure on the collection surface to localize, differentiate and enumerate peripheral blood selected epithelial derived tumor cells. In this example, 5 ml of blood was incubated with 35 μg of an epithelial cell specific ferrofluid (EPCAM-FF, Immunicon Corp., Huntingdon Valley, PA) for 15 minutes. The sample was placed in a quadrupole magnetic separator (QMS 17, Immunicon Corp.) for 10 minutes and the blood was discarded. The vessel was taken out of the separator and the collected cells present at the wall of the separation vessel were resuspended in a 3 ml of a buffer containing a detergent to permeabilize the cells (Immunoperm, Immunicon Corp.) and placed back in the separator for 10 minutes. The buffer containing the detergent was discarded and the vessel was taken out of the separator and the cells collected at the wall were resuspended in 200 μl of a buffer containing the UV excitable nucleic acid dye DAPI (Molecular Probes) and Cytokeratin monoclonal antibodies (identifying epithelial cells) labeled with the fluorochrome Cy3. The cells were incubated for 15 minutes after which the vessel was placed in the separator. After 5 minutes the uncollected fraction containing excess reagents was discarded, the vessel was taken out of the separator and the collected cells were resuspended in 200 μl of an isotonic buffer. This solution was placed into a collection chamber and placed in the magnetic separator shown in FIG. 1A. The ferrofluid labeled cells and the free ferrofluid particles moved immediately to the collection surface and were evenly distributed along the surface as is shown in FIG. 2A. The figure shows a representative area on the collection surface using transmitted light and a 20X objective. In FIG. 2B the same field is shown but now a filter cube is used for Cy3 excitation and emission. Two objects can be identified and are indicated with 1 and 2. FIG. 2C shows the same field but the filter cube is switched to one with an

excitation and emission filter cube for DAPI. The objects at position 1 and 2 both stain with DAPI as indicated at positions 3 and 5 confirm their identity as epithelial cells. Additional non epithelial cells and other cell elements cells are identified by the DAPI stain; an example is indicated by the number 4.

II. V-shaped grooved as collection structures

To provide for spatially patterned collection of target specimens for qualitative and quantitative analysis of microscopic biologic samples, the present invention relates to making and using V-groove structures on the inner surface of the imaging chamber. Generally, V-grooves are long v-shaped grooves, pre-molded into the inner portion of the viewing surface on the imaging chamber. These structures provide an alignment of cells as good as or even better than previously reported Ni lines. Furthermore, V-grooves are made from a highly transparent material, optically suited for imaging the entire cell. A schematic drawing of the V-grooves together with the alignment principle of the Ni lines, for comparison, is shown in Figure 3.

Figure 3 illustrates the principle of cell alignment using V-grooves. Magnetically induced cell movement in the chamber is similar to Ni lines, except at the inner surface of the sample chamber. Here, the magnetically labeled cells will either collide with the inclined surface of the V-grooves and slide into the top of the groove (indicated in the above Figure by L), or they will directly hit the top of the V-groove. In either situation, the cells will align in the groove, allowing for subsequent imaging.

In order for sufficient movement along the inclined surface of the groove, the surface should be flat and cells prohibited from sticking to the walls. To achieve a smooth precise V-groove design, known wafer etching technologies are used. However because of expense and optical requirements, silicon wafers are not appropriate, rather polydimethylsiloxane (PDMS) replica molding provides a composition that will meet these requirements. Compositions that will meet this criteria are also considered in the present invention. V-grooves, etched onto a silicon wafer, are the inverse of the eventual design, and provide the PDMS mold with the correct V-groove shape when poured onto the silicon mold. After curing, this shape is cut into dimensions that would allow replacement of the glass surface of the imaging chamber.

III. Longitudinal Variation of Chamber Height

The height of the chamber in concert with the concentration of the target entity determines the density of the distribution of target specimens collected at the collection surface of a vessel such as described above. To increase the range of surface collection densities which are acceptable for accurate counting and analysis, one can vary the height of the chamber to eliminate the need to dilute or concentrate the sample, for analysis of samples where the concentration may vary widely. In FIG. 4A, a cross section of a chamber is shown with a

collection surface 1, and six compartments having different heights. Target cells are randomly positioned in the chamber. In FIG. 4B the same cross section is shown but now the cells have moved to the collection surface under the influence of the magnetic gradient. In the area of the highest chamber depth, the density of the cells is too high to be accurately measured, whereas in the area of the lowest chamber depth, too few cells are present to provide an accurate cell count. To further illustrate this principle, a histogram of the cell density along the collection surface is shown in FIG. 4C. Note that the number of cells in the area with the highest density is underestimated. The approach described here increases the range of concentrations which can be accurately measured as compared to the cell number measurements traditionally used in hematology analyzers and flow cytometers.

IV. Wafer Etching and PDMS Molding on Inner Surface of Viewing Face of Chamber

Etching can be accomplished on any optically transparent material that can be used in the manufacture of the chamber. By example, silicon wafers can be used in etching because of the ease of precision, fine detail, and reproducability. Any material with similar characteristics and known in the art is considered in the present invention. Etching of the V-groove shapes uses two common etching techniques. First an etch mask that is needed to etch the grooves is created. This mask is created using BHF (Buffered Hydrofluoric acid) etching. The process of BHF etching is explained in Figure 5. Once BHF etching is complete, thin layers of SiO_2 are left on the silicon wafer at places where no V-groove should be etched. Anisotropic etching is also used to etch the V-grooves. Here, KOH is used as etchant. When this process is applied to a properly orientated wafer, V-grooves are etched, limited by the crystal plane of the silicon wafer. Accordinly, a highly reproducible and constant etch angle is produced. The angle depends on the wafer orientation with one embodiment at a constant 35.26 degrees. Another technique is Deep Reactive Ion Etching (DRIE). By using this technique it is possible to etch structures with a high aspect ratio (ratio between length and width of the structure). DRIE cyclically alternates between etch and deposition steps forming scalloped sidewalls.

PDMS molding is used to obtain a positive imprint on the fabricated wafer. PDMS or Polydimethylsiloxane (Dow Corning (Sylgard 184, Dow Corning, Midland, MI, USA) is a polymer containing the siloxane bond between Si (Silicon) and O (Oxygen). The polymers molecules are linked together to form longer polymers with an average number around 50 to 100.

The final PDMS is obtained with the addition of a cross-linker. The cross-linker connects with the polymers to form long networks of polymers, resulting in a clear, elastic, chemically inert, thermally stable material. After polymerization, the PDMS forms a clear flexible substance which adheres to very few materials and is not hygroscopic, thus preventing any

sticking of cells to the sides due to the fact that PDMS adheres to very few materials. Furthermore, it is thermally stable and transparent from approximately 300 to 900 nm. These characteristics are all important for its use in a fluorescent imaging system and the transmission of visible light. Figure 6 illustrates the relationship between the wafer, PDMS mold and the formation of the V-grooves. After formation the V-grooves are cut into the dimensions of the viewing face of the chamber. Figure 7 depicts the transmission spectrum through the viewing surface.

V. Parameters of the V-groove viewing surface and examples of use

The prameters considered are shown in Figure 8. L is the width of the flat horizontal area and D is the spacing of the grooves. Varying L will influence the alignment of the cells in the groove. If L is too big, cells may overlap or may be not perfectly aligned in the center. Misalignment influences scanning and imaging, complicating subsequent image analysis. As a consequence, the size of the laser spot has to be increased so as to match the increased area that has to be illuminated. The spacing of the grooves is controlled by D. This influences the maximum cell size and the number of cells that can be accommodated.

One possible example of a wafer design incorporates a chimney-like design (Figure 9). This design accommodates the excess ferrofluid in solution to a position away from the cells. This design were fabricated using DRIE high aspect ratio etching. The width of the chimneys should be smaller than the smallest diameter of an interested cell.

An example to depict the quality with which CTC's are imaged is demonstrated with Hela cells. Hela cells are labeled with Cytokeratin-PE (Figure 10A) and DAPI (Figure 10B) to fluorescently stain the nucleus and the cytoskeleton. These cells were tested in a chamber fitted with a V-groove structure on the viewing surface. Cells labeled with both cytokeratin-PE and DAPI were imaged at several focal points along the V-groove. At 200 μm, the top of the V-groove is in focus. Lower values indicate a lower point of focus.

CLAIMS

1. An improved method for optically analyzing microbiological specimens suspended in a fluid medium by magnetically labeling said specimens, which method comprises containing said magnetically-labeled specimens in a chamber having an optically-transparent viewing face, positioning said chamber into a magnetic field having a substantially uniform region of vertically-directed magnetic gradient and such that said chamber is located in said uniform region, collecting a uniformly-distributed layer of said magnetically-labeled specimens on the interior surface of said viewing face of said chamber, and conducting optical anlaysis of said magnetically-labeled specimens while maintaining said magnetically-labeled specimens collected on said interior surface of said chamber, and wherein said improvement comprises collecting said uniformly-distributed layer of said magnetically-labeled specimens within preformed V-grooves on said inner surface of said optically-transparent face of said chamber.
2. The method of claim 1 wherein said V-grooves contain a chimney-shaped component in order to allow unbound magnetic label to partition above a focal plane for said optical analysis.
3. The method of claim 1 wherein said fluid medium containing said magnetically-labeled specimens has a predetermined dilution of said magnetically-labeled specimens such that said dilution provides for optimum alignment of said magnetically-labeled specimens along inner surface of viewing face.
4. The method of claim 1 wherein said specimens is from a group consisting of epithelial cells, ciruculating tumor cells, endothelial cells, fungal cells, bacterial cells, and combinations thereof.
5. An improved apparatus for observing magnetically responsive microscopic specimens suspended in a fluid member, said apparatus having a fluid containing chamber with an optically-transparent face, a ferromagnetic capture structure supported on the interior surface of said transparent face, magnetic means for inducing an internal magnetic gradient in the vicinity of said ferromagnetic capture structure, whereby said magnetically responsive specimens are immobilized along said face adjacent to said capture structure and an electrical conductor means supported on said transparent wall for enabling electrical manipulation of said immobilized specimens, wherein said improvement comprises a collection means having preformed V-grooves on the inner surface of said optically-transparent face to allow for uniform distribution of said specimens during optical analysis.

6. The improved apparatus of claim 5 wherein said V-grooves contain chimney-shaped components for separating small magnetically responsive entities from larger magnetically reponsive specimens during optical analysis.
7. The improved apparatus of claim 5 wherein said specimen is from a group consisting of epithelial cells, ciruculating tumor cells, endothelial cells, fungal cells, bacterial cells and combinations thereof.
8. An apparatus for observing magnetically responsive microbiological specimens suspended in a fluid member, comprising:
    a. a fluid containing chamber with an optically-transparent face;
    b. a ferromagnetic capture structure supported on the interior surface of said transparent face;
    c. collection means having a preformed inner surface topography of said optically-transparent face to allow for uniform distribution of said specimens during optical analysis.
9. The apparatus of claim 8 whereby said inner surface contains V-grooves for optimum alignment of said specimens during optical analysis.
10. The apparatus of claim 9 whereby said V-grooves contain chimney-shaped components for separating small magnetically responsive entities from larger magnetically reponsive specimens during optical analysis.
11. The improved apparatus of claim 5 wherein said specimens is from a group consisting of epithelial cells, ciruculating tumor cells, endothelial cells, fungal cells, bacterial cells, and combinations thereof.

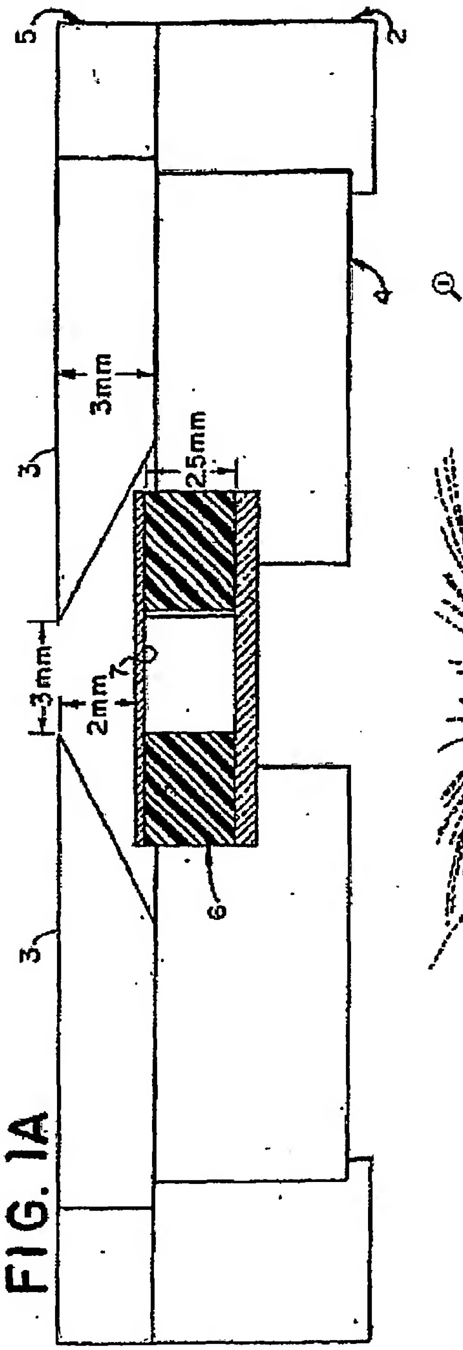


FIG. 1A

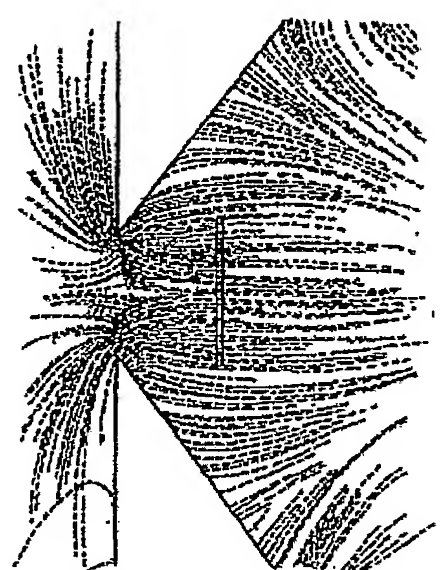


FIG. 1B

FIG. 2A

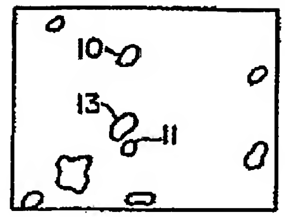


FIG. 2B

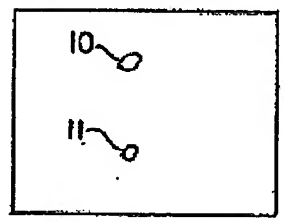


FIG. 2C

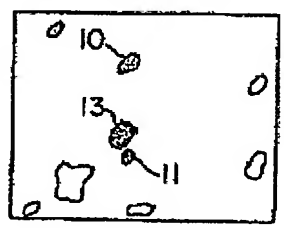


FIGURE 3

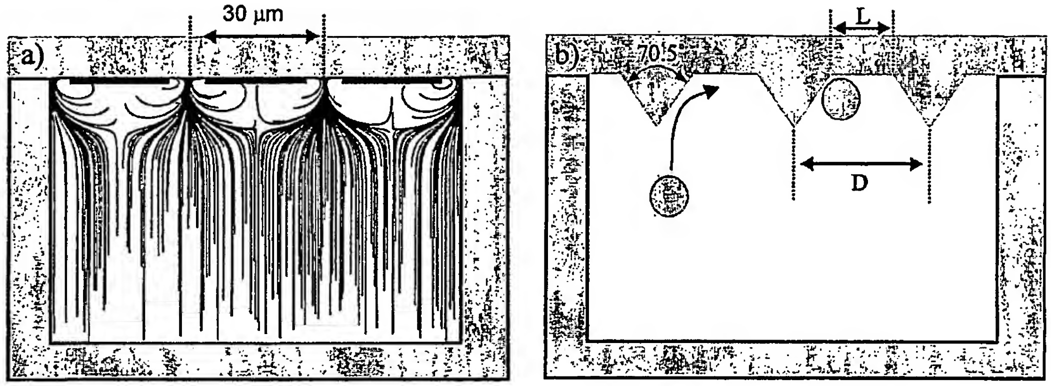


FIGURE 4

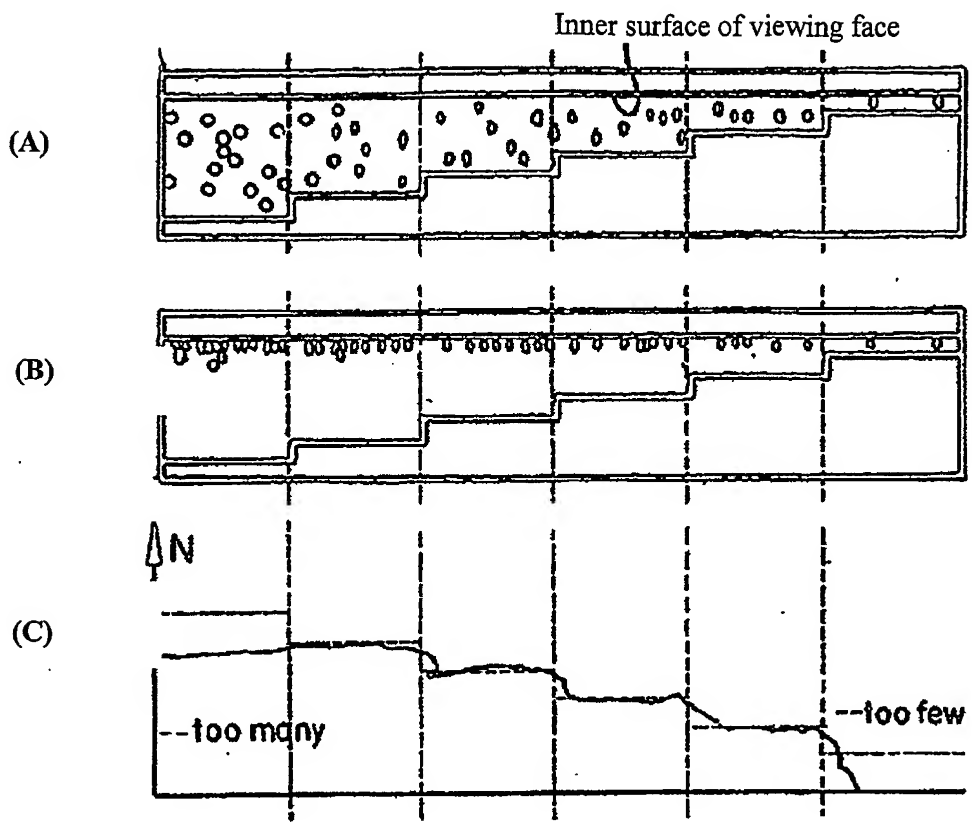


FIGURE 5

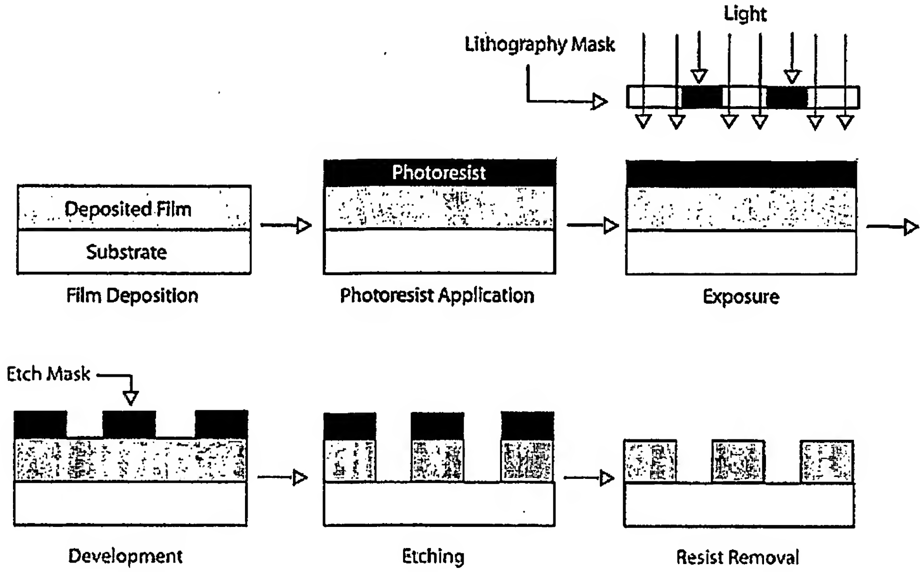
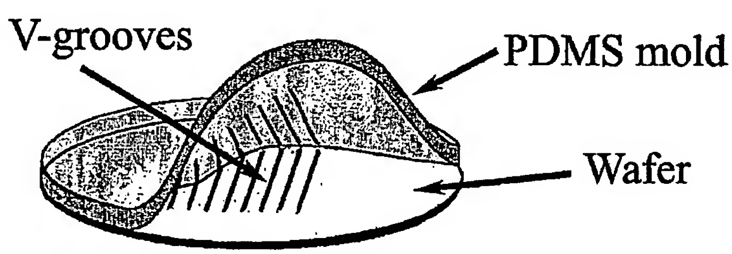
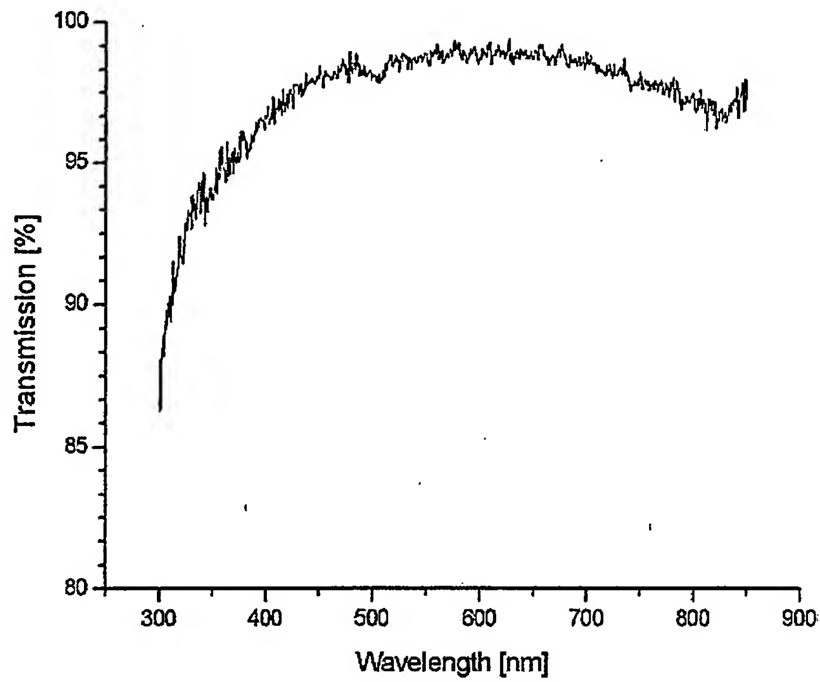


FIGURE 6




100
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400
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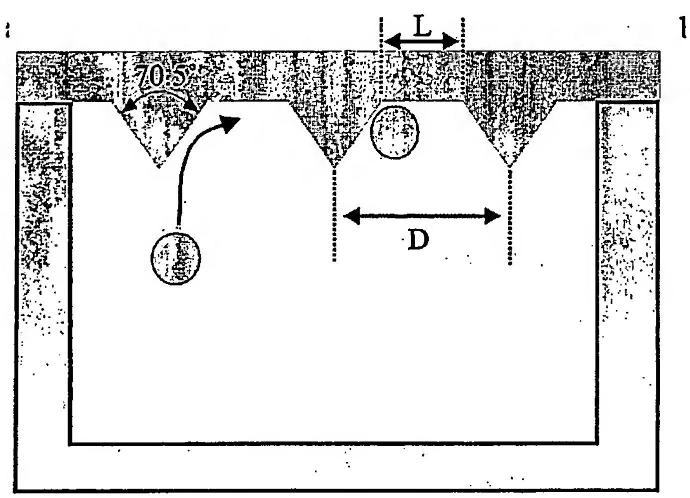
Wavelength [nm]

FIGURE 8


L
1
70.5°
D

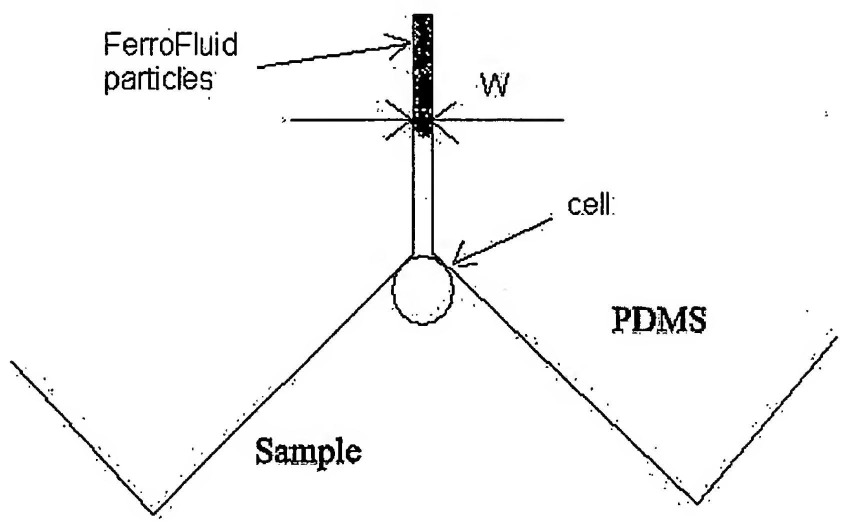
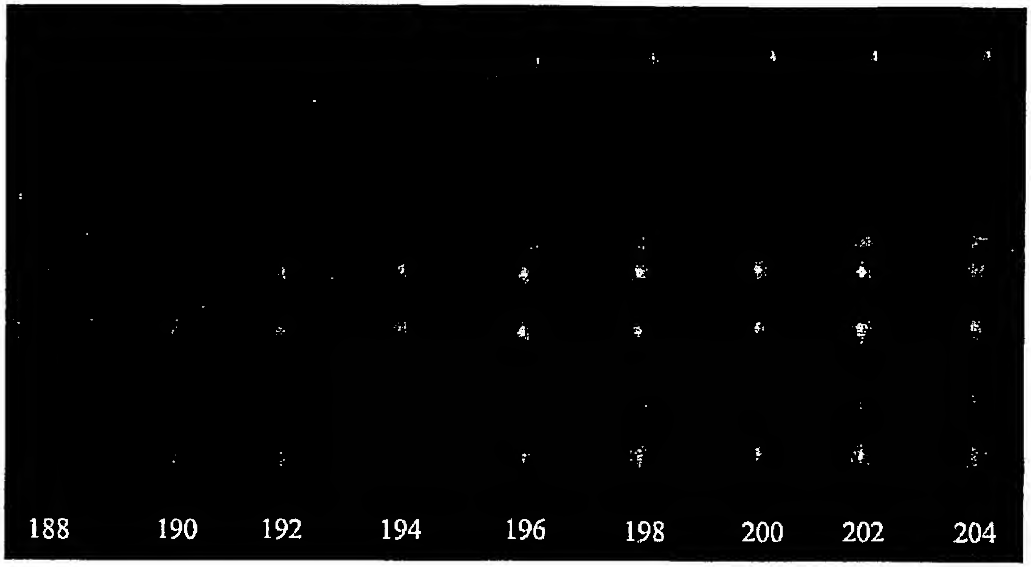

FerroFluid
particles
W
cell
PDMS
Sample

FIGURE 10

(A)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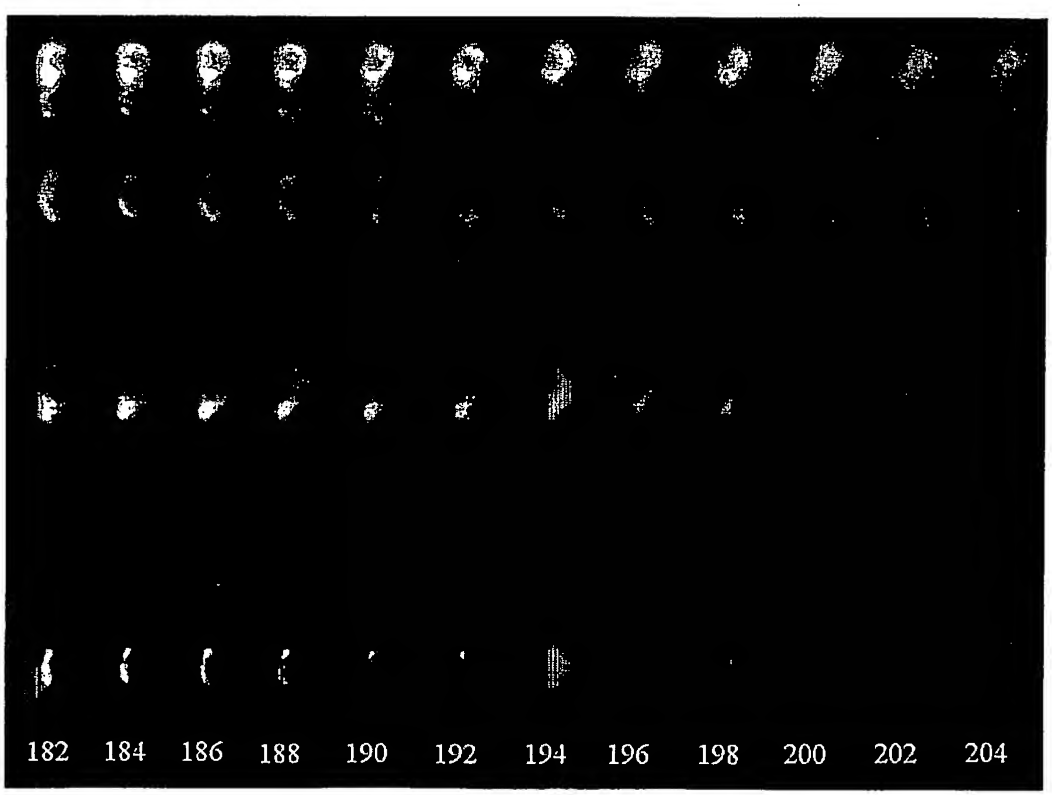
(B)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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/31132

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : BO1D 35/06; GO1N 33/533
US CL : 210/695,94,222; 436/177,526; 435/7.2; 209/213,214,223
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 6,790,366 B2 (TERSTAPPEN et al) 14 September 2004 (14.09.2004), entire document.	1-11
A	US 6,013,532 A (LIBERTI et al) 11 January 2000 (11.01.2000), entire document.	1-11
A	US 5,985,153 A (DOLAN et al) 16 November 1999 (16.11.1999), entire document.	1-11
A	US 5,411,863 A (MILTENYI) 02 May 1995 (02.05.1995), entire document.	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search	Date of mailing of the international search report
04 December 2004 (04.12.2004)	16 DEC 2004

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	David A Reifsnyder Telephone No. (571) 272-1700

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(54) **Title:** MAGNETIC DEVICE FOR ISOLATION OF CELLS AND BIOMOLECULES IN A MICROFLUIDIC ENVIRONMENT

(57) **Abstract:** The present invention features a new and useful magnetic device and methods of its use for isolation, enrichment, and purification of cells, proteins, DNA, and other molecules. In general the device includes magnetic regions or obstacles to which magnetic particles can bind. The chemical groups, i.e., capture moieties, on the surface of the magnetic particles may then be used to bind particles, e.g., cells, or molecules of interest from complex samples, and the bound species may then be selectively released for downstream collection or further analysis.

MAGNETIC DEVICE FOR ISOLATION OF CELLS AND BIOMOLECULES IN A MICROFLUIDIC ENVIRONMENT

BACKGROUND OF THE INVENTION

The invention relates to the fields of microfluidics and sorting of particles and molecules.

There are several approaches devised to separate a population of homogeneous cells from complex mixtures, such as blood. These cell separation techniques may be grouped into two broad categories: (1) invasive methods based on the selection of cells fixed and stained using various cell-specific markers; and (2) noninvasive methods for the isolation of living cells using a biophysical parameter specific to a population of cells of interest.

Invasive techniques include fluorescence activated cell sorting (FACS), magnetic activated cell sorting (MACS), and immunomagnetic colloid sorting. FACS is usually a positive selection technique that uses a fluorescently labeled marker to bind to cells expressing a specific cell surface marker. FACS can also be used to permeabilize and stain cells for intracellular markers that can constitute the basis for sorting. It is fast, typically running at a rate of 1,000 to 1,500 Hz, and well established in laboratory medicine. High false positive rates are associated with FACS because of the low number of photons obtained during extremely short dwell times at high speeds. Complicated multiparameter classification approaches can be used to enhance the specificity of FACS, but multianalyte-based FACS may be impractical for routine clinical testing because of the high cost associated with it. The clinical application of FACS is further limited because it requires considerable operator expertise, is laborious, results in cell loss due to multiple manipulations, and the cost of the equipment is prohibitive.

MACS is used as a cell separation technique in which cells that express a specific surface marker are isolated from a mixture of cells using magnetic beads coated with an antibody against the surface marker. MACS has the advantage of being cheaper, easier, and faster to perform as compared with FACS. It suffers from cell loss due to multiple manipulations and handling.

A magnetic colloid system has been used in the isolation of cells from blood. This colloid system uses ferromagnetic nanoparticles that are coated with goat anti-mouse IgG that can be easily attached to cell surface antigen-specific monoclonal antibodies. Cells that are labeled with ferromagnetic nanoparticles align in a magnetic field along ferromagnetic Ni lines deposited by lithographic techniques on an optically transparent surface. This approach also requires multiple cell handling steps including mixing of cells with magnetic beads and separation on the surfaces. It is also not possible to sort out the individual cells from the sample for further analysis.

Noninvasive techniques include charge flow separation, which employs a horizontal crossflow fluid gradient opposing an electric field in order to separate cells based on their characteristic surface charge densities. Although this approach can separate cells purely on biophysical differences, it is not specific enough. There have been attempts to modify the device characteristics (e.g., separator screens and buffer counterflow conditions) to address this major shortcoming of the technique. None of these modifications of device characteristics has provided a practical solution given the expected individual variability in different samples.

Since the prior art methods suffer from high cost, low yield, and lack of specificity, there is a need for a method for depleting a particular type of cell from a mixture that overcomes these limitations.

SUMMARY OF THE INVENTION

The present invention features a new and useful magnetic device and methods of its use for isolation, enrichment, and purification of cells, proteins, DNA, and other molecules. In general the device includes magnetic regions or obstacles to which magnetic particles can bind. The chemical groups, i.e., capture moieties, on the surface of the magnetic particles may then be used to bind particles, e.g., cells, or molecules of interest from complex samples, and the bound species may then be selectively released for downstream collection or further analysis.

In one aspect, the invention features a device for the separation of one or more desired analytes from a sample. The device includes a first region of magnetic obstacles disposed in a channel, e.g., a microfluidic channel, and a plurality of magnetic particles attached to at least one of the obstacles by a magnetic interaction.

Another device of the invention for the separation of one or more desired analytes from a sample includes a channel having a plurality of magnetic obstacles, wherein the obstacles include a plurality of magnetic particles, e.g., without any underlying support structure, and a capture moiety capable of binding the one or more analytes is attached to the particles. Alternatively, a device for the separation of one or more desired analytes from a sample includes a channel having a plurality of magnetic obstacles, wherein the obstacles include a plurality of magnetic particles, and the magnetic obstacles are disposed such that at least a portion of the one or more analytes cannot pass between the obstacles. In these embodiments, the channel may further include a region of a plurality of magnetic locations, where the magnetic obstacles are attached to the locations by a magnetic interaction.

In any of the above devices, the obstacles are typically ordered in a two-dimensional array, but can also be randomly disposed. The device may further include a second region of magnetic obstacles, e.g., made of a plurality of magnetic particles, or having a plurality of magnetic particles attached by magnetic interaction thereto. The first and second regions can be arranged in series, in parallel, or interspersed. In some embodiments, a capture moiety capable of binding, specifically or not, one or more analytes is attached to the magnetic particles. Exemplary capture moieties include holo-transferrin and an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, and combinations thereof. When two or more regions of obstacles are employed, different regions may contain different capture moieties to bind two or more different analytes. When capture moieties are employed, the obstacles are typically disposed such that the one or more analytes are capable of passing between the obstacles. When capture moieties are not employed, the obstacles may be disposed such that at least a portion of the one or more analytes cannot pass between the obstacles, e.g., based on size, shape, or deformability.

Other compounds, e.g., cell surface receptors and candidate drug compounds, may also be attached to a magnetic particle, with or without a capture moiety. The attachment of other compounds to magnetic particles allows for the determination of the effect of that compound on an analyte, e.g., effects of candidate drugs on cells, or the identification of ligands for cell surface receptors. The attachment of a plurality of candidate drug compounds or receptors allows for high throughput screening in the device.

In other embodiments, at least a portion of the magnetic obstacles includes a permanent or non-permanent magnet. A device may also include a magnetic force generator capable of producing a magnetic field in the magnetic obstacles, e.g., an electromagnetic or a permanent magnet having a nonuniform magnetic

field. Preferably, the magnetic field generator is capable of independently applying the magnetic field to one or more obstacles.

The invention also features a method for retaining a first type of analyte in a sample including providing a sample containing at least a first and a second type of analyte and a device of the invention and introducing the sample into the device, wherein the first type of analyte is retained in the device, e.g., by binding to a capture moiety or being retained based on size, shape, or deformability. Preferably, at least 60% of analytes of the first type in the sample are retained, and at least 70% of analytes of the second type in the sample are not retained. The method may also be altered to retain a third type of analyte in the device as well. Once retained, analytes may be contacted with a labeling moiety. The retained analytes may also be released from the device, e.g., for collection, culturing, or analysis, by interrupting the magnetic interaction holding the magnetic particles in the device, or by disrupting an interaction between the analyte and a capture moiety or the capture moiety and the magnetic particle. When a candidate drug compound is attached to the magnetic particles, the first type of analyte is typically a cell, and the method may further include determining the effect of the candidate drug compound on the cell. Similar methods can be used when cell surface receptors are bound to the magnetic particles as the capture moiety, and putative ligands, agonists, or antagonists are the analytes.

By "analyte" is meant a molecule, other chemical species, e.g., an ion, or particle. Exemplary analytes include cells, viruses, nucleic acids, proteins, carbohydrates, and small organic molecules.

By "capture moiety" is meant a chemical species to which a particle binds. A capture moiety may be a compound coupled to a surface or the material making up the surface. Exemplary capture moieties include antibodies, oligo- or polypeptides, nucleic acids, other proteins, synthetic polymers, and carbohydrates.

By "diluent" is meant any fluid that is miscible with the fluid medium of a sample. Typically diluents are liquids. A diluent, for example, contains agents to alter pH (e.g., acids, bases, or buffering agents) or reagents to chemically modify analytes in a sample (e.g., to label an analyte, conjugate a chemical species to an analyte, or cleave a portion of an analyte) or to effect a biological result (e.g., growth media or chemicals that elicit a cellular response or agents that cause cell lysis). A diluent may also contain agents for use in fixing or stabilizing cells, viruses, or molecules. A diluent may also be chemically or biologically inert.

By "magnetic" is meant possessing hard (permanent) or soft (non-permanent) magnetic properties.

By "microfluidic" is meant having at least one dimension of less than 1 mm. For example, a microfluidic device includes a microfluidic channel having a height, width, or length of less than 1 mm.

By "obstacle" is meant an impediment to flow in a channel, e.g., a protrusion from one surface.

By "particle" is meant an object that does not dissolve in a solution on the time scale of an analysis.

By "type" of analyte is meant a population of analytes, e.g., cells or molecules, having a common property, e.g., the presence of a particular surface antigen. A single analyte may belong to several different types of analytes.

By "specifically binding" a type of analyte is meant binding analytes of that type by a specified mechanism, e.g., antibody-antigen interaction, ligand-receptor interaction, nucleic acid complementarity, protein-protein interaction, charge-charge interaction, and hydrophobic-hydrophobic or hydrophilic-hydrophilic interactions. The strength of the bond is generally enough to prevent detachment by the flow of fluid present when analytes are bound, although individual analytes may occasionally detach under normal operating conditions.

Advantages of the invention include the ability to provide a sorting device that need not be functionalized with environmentally sensitive capture moieties prior to packaging the device, thereby increasing the bandwidth of usable capture moieties; a sorting device that can be functionalized with the capture molecules by the end-user in a simple, rapid and reliable manner enabling customized devices for end-user specific applications; and a sorting device that is more universally functional than the prior art devices.

Other features and advantages will be apparent from the following description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a cross-sectional view of a device of the invention and associated process flow for cell isolation followed by release for off-line analysis according to the present invention.

FIG. 2 is a schematic of the fabrication and functionalization of a device of the invention. The magnetized posts enable post-packaging modification of the device.

FIG. 3 is a schematic of an application of a device of the invention to capture and release CD71+ cells from a complex mixture, such as blood, using monoclonal antibodies to the transferrin (CD71) receptor.

FIG. 4 is a schematic representation of an application of a device of the invention to capture and release CD71+ cells from a complex mixture, such as blood, using holo-transferrin. Holo-transferrin is rich in iron content, commercially available, and has higher affinity constants and specificity of interaction with the CD71 receptor than its counterpart monoclonal antibody.

DETAILED DESCRIPTION OF THE INVENTION

Device

The invention features a device, typically microfluidic, containing a plurality of magnetic obstacles. In its simplest embodiment, the device includes a channel having magnetic regions to which magnetic particles can magnetically attach to create a textured surface, with which analytes passing through the channel can come into contact. By coating these magnetic particles with appropriate capture moieties it is possible to bind desired analytes through affinity mechanisms. The magnetic particles can serve to texture the channel, and through the appropriate choice of magnetic particle size and shape relative to the dimensions of the channel, it is possible to provide a texture that enhances interactions between the analytes of interest and the magnetic particles. The magnetic particles can be magnetically attached to hard magnetic regions of the channel or to soft magnetic regions that are actuated to produce a magnetic field. In addition, these magnetic particles can be released from defined locations within the channel, e.g., by increasing the overall flow rate of the fluid flowing through the device, decreasing the magnetic field, or through some combination of the two. In one embodiment, a spatially nonuniform permanent magnet or electromagnet may be used to create organized and in some cases periodic arrays of magnetic particles within an otherwise untextured microfluidic channel (Deng et al. Applied Physics Letters, 78, 1775 (2001)). An electromagnetic may be employed to create a non-uniform magnetic field in a device. The non-uniform filed creates regions of higher and lower magnetic field strength, which, in turn, will attract magnetic particles in a periodic arrangement within the device. Other external magnetic fields may be employed to create magnetic regions to which magnetic particles attach. A hard magnetic material may also be used in the fabrication of the device, thereby obviating the need for electromagnets or external magnetic fields. In one

embodiment, the device contains a plurality of channels having magnetic regions, e.g., to increase volumetric throughput. Further, these channels may be stacked vertically.

FIGURE 1 illustrates an exemplary device geometry and functional process flow to isolate and then release target analytes, e.g., cells or molecules, from a complex mixture. The device contains obstacles that extend from one channel surface toward the opposing channel surface. The obstacles may or may not extend the entire distance across the channel. The obstacles are magnetic (e.g., contain hard or soft magnetic materials or are locations of high magnetic field in a non-uniform field) and attract and retain magnetic particles, which are typically coated with capture moieties. The device geometry, the distribution, shape, size of the posts and the flow parameters can be altered to optimize the efficiency of the interaction of the analytes of interest with the capture moieties (e.g., as described in International Application No. PCT/US03/30965). In one specific example, an anodic lidded silicon wafer with microtextured magnetic obstacles of varying shapes (cylindrical, rectangular, trapezoidal, or pleomorphic) and size (10 – 999 microns) are arranged uniquely (spacing and density varied across equilateral triangular, diagonal, and random array distribution) to maximize the collision frequency of analytes with the obstacles within the confines of a continuous perfusion flow stream. The exact geometry of the magnetic obstacles and the distribution of obstacles may depend on the type of analytes being isolated, enriched, or purified.

Devices of the invention may or may not include microfluidic channels, i.e., may or may not be microfluidic devices. The dimensions of the channels of the device into which a sample is introduced may depend on the sample employed. Preferably, a channel has at least one dimension (e.g., height, width, length, or radius) of no greater than 10, 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 6.5, 5, 4.5, 4, 3.5, 3, 2.5,

2, 1.5, or 1 mm. Microfluidic devices described herein preferably have channels having at least one dimension of less than 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, or even 0.05 mm. The dimensions of the channels can be determined by one skilled in the art based on the desired application.

Fabrication

A variety of techniques can be employed to fabricate a device of the invention, and the technique employed will be selected based in part on the material of choice. Exemplary materials for fabricating the devices of the invention include glass, silicon, steel, nickel, other metals, poly(methylmethacrylate) (PMMA), polycarbonate, polystyrene, polyethylene, polyolefins, silicones (e.g., poly(dimethylsiloxane)), ceramics, and combinations thereof. Other materials are known in the art. Methods for fabricating channels in these materials are known in the art. These methods include, photolithography (e.g., stereolithography or x-ray photolithography), molding, embossing, silicon micromachining, wet or dry chemical etching, milling, diamond cutting, Lithographie Galvanoformung and Abformung (LIGA), and electroplating. For example, for glass, traditional silicon fabrication techniques of photolithography followed by wet (KOH) or dry etching (reactive ion etching with fluorine or other reactive gas) can be employed. Techniques such as laser micromachining can be adopted for plastic materials with high photon absorption efficiency. This technique is suitable for lower throughput fabrication because of the serial nature of the process. For mass-produced plastic devices, thermoplastic injection molding, and compression molding is suitable. Conventional thermoplastic injection molding used for mass-fabrication of compact discs (which preserves fidelity of features in sub-microns) may also be employed to fabricate the devices of the invention. For example, the device features are replicated on a glass master

by conventional photolithography. The glass master is electroformed to yield a tough, thermal shock resistant, thermally conductive, hard mold. This mold serves as the master template for injection molding or compression molding the features into a plastic device. Depending on the plastic material used to fabricate the devices and the requirements on optical quality and throughput of the finished product, compression molding or injection molding may be chosen as the method of manufacture. Compression molding (also called hot embossing or relief imprinting) has the advantages of being compatible with high-molecular weight polymers, which are excellent for small structures, but is difficult to use in replicating high aspect ratio structures and has longer cycle times. Injection molding works well for high-aspect ratio structures but is most suitable for low molecular weight polymers.

A device may be fabricated in one or more pieces that are then assembled. Pieces of a device may be bonded together by clamps, adhesives, heat, anodic bonding, or reactions between surface groups (e.g., wafer bonding). Alternatively, a device may be fabricated as a single piece, e.g., using stereolithography or other three-dimensional fabrication techniques.

Magnetic regions of the device can be fabricated with either hard or soft magnetic materials, such as, but not limited to, rare earth materials, neodymium-iron-boron, ferrous-chromium-cobalt, nickel-ferrous, cobalt-platinum, and strontium ferrite. Portions of the device may be fabricated directly out of magnetic materials, or the magnetic materials may be applied to another material. The use of hard magnetic materials can simplify the design of a device because they are capable of generating a magnetic field without other actuation. Soft magnetic materials, however, enable release and downstream processing of bound analytes simply by demagnetizing the material. Depending on the magnetic material, the application process can include cathodic sputtering, sintering,

electrolytic deposition, or thin-film coating of composites of polymer binder-magnetic powder. A preferred embodiment is a thin film coating of micromachined obstacles (e.g., silicon posts) by spin casting with a polymer composite, such as polyimide-strontium ferrite (the polyimide serves as the binder, and the strontium ferrite as the magnetic filler). After coating, the polymer magnetic coating is cured to achieve stable mechanical properties. After curing, the device is briefly exposed to an external induction field, which governs the preferred direction of permanent magnetism in the device. The magnetic flux density and intrinsic coercivity of the magnetic fields from the posts can be controlled by the % volume of the magnetic filler.

In another embodiment, an electrically conductive material is micropatterned on the outer surface of an enclosed microfluidic device. The pattern may consist of a single, electrical circuit with a spatial periodicity of approximately 100 microns. By controlling the layout of this electrical circuit and the magnitude of the electrical current that passes through the circuit, one can develop periodic regions of higher and lower magnetic strength within the enclosed microfluidic device.

The magnetic particles can be disposed uniformly throughout a device or in spatially resolved regions. In addition, magnetic particles may be used to create structure within the device. For example, two magnetic regions on opposite sides of a channel can be used to attract magnetic particles to form a "bridge" linking the two regions.

The magnetic field can be adjusted to influence supra and paramagnetic particles with magnetic mass susceptibility ranging from $0.1 - 200 \times 10^{-6}$ m^3/kg. The paramagnetic particles of use can be classified based on size: *particulates* (1 – 5 μm in the size of a cell diameter); *colloidal* (on the order of 100 nm); and

molecular (on the order of 2-10 nm). The fundamental force acting on a paramagnetic entity is:

$$F_b = \frac{1}{2\mu_o} \Delta\chi V_G \nabla B^2$$

where F_b is the magnetic force acting on the paramagnetic entity of volume V_b, $\Delta\chi$ is the difference in magnetic susceptibility between the magnetic bead, χb, and the surrounding medium, χf, μ_o is the magnetic permeability of free space, B is the external magnetic field, and ∇ is the gradient operator. The magnetic field can be controlled and regulated to enable attraction and retention of a wide spectrum of particulate, colloidal, and molecular paramagnetic entities typically coupled to capture moieties.

Magnetic Particles and Capture Moieties

Any magnetic particles that respond to a magnetic field may be employed in the devices and methods of the invention. Desirable particles are those that have surface chemistry that can be chemically or physically modified, e.g., by chemical reaction, physical adsorption, entanglement, or electrostatic interaction.

Capture moieties can be bound to magnetic particles by any means known in the art. Examples include chemical reaction, physical adsorption, entanglement, or electrostatic interaction. The capture moiety bound to a magnetic particle will depend on the nature of the analyte targeted. Examples of capture moieties include, without limitation, proteins (such as antibodies, avidin, and cell-surface receptors), charged or uncharged polymers (such as polypeptides, nucleic acids, and synthetic polymers), hydrophobic or hydrophilic polymers, small molecules (such as biotin, receptor ligands, and chelating agents), and ions. Such capture moieties can be used to specifically bind cells (e.g., bacterial, pathogenic, fetal cells, fetal blood cells, cancer cells, and blood cells), organelles (e.g., nuclei),

viruses, peptides, protein, polymers, nucleic acids, supramolecular complexes, other biological molecules (e.g., organic or inorganic molecules), small molecules, ions, or combinations or fragments thereof. Specific examples of capture moieties include antiCD71, antiCD36, antiGPA, and holotransferrin. In another embodiment, the capture moiety is fetal cell specific.

Applications

The methods of the invention involve contacting an analyte, for example as a part of a mixture, with the surfaces of a device, and desired analytes (e.g., rare cells such as fetal cells, pathogenic cells, cancer cells, or bacterial cells) in a sample are retained in the device. Analytes of interest may then bind to the surfaces of the device. In another embodiment, desired analytes are retained in the device through size-, shape- or deformability-based separation. Desirably, at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% of the desired analytes are retained in the device. The surfaces of the device are desirably designed to minimize nonspecific binding of non-target analytes. For example, at least 99%, 98%, 95%, 90%, 80%, or 70% of non-target analytes are not retained in the device. The selective retention in the device can result in the separation of a specific analyte population from a mixture, e.g., blood, sputum, urine, and soil, air, or water samples.

The selective retention of desired analytes is obtained by introduction of magnetic particles into a device of the invention. Capture moieties may be bound to the magnetic particles to effect specific binding of the target analyte. Alternatively, the magnetic particles may be disposed such as to only allow analytes of a selected size, shape, or deformability to pass through the device. Combinations of these embodiments are also envisioned. For example, a device may be configured to retain certain analytes based on size and others based on

binding. In addition, a device may be designed to bind more than one analyte of interest, e.g., in a serial, parallel, or interspersed arrangement of regions within the device or where two or more capture moieties are disposed on the same magnetic particle or on adjacent particles, e.g., bound to the same obstacle or region. Further, multiple capture moieties that are specific for the same analytes (e.g., antiCD71 and antiCD36) may be employed in the device, either on the same or different magnetic particles, e.g., disposed on the same or different obstacle or region.

Magnetic particles may be attached to obstacles present in the device (or manipulated to create obstacles) to increase surface area for analytes to interact with to increase the likelihood of binding. The flow conditions are typically such that the analytes are very gently handled in the device to prevent damage. Positive pressure or negative pressure pumping or flow from a column of fluid may be employed to transport analytes into and out of the microfluidic devices of the invention. The device enables gentle processing, while maximizing the collision frequency of each analyte with one or more of the magnetic particles. The target analytes interact with any capture moieties on collision with the magnetic particles. The capture moieties can be co-localized with obstacles as a designed consequence of the magnetic field attraction in the device. This interaction leads to capture and retention of the target analytes in defined locations. Alternatively, analytes are retained based on an inability to pass through the device, e.g., based on size, shape, or deformability. Captured analytes can be released by demagnetizing the magnetic regions retaining the magnetic particles. For selective release of analytes from regions, the demagnetization can be limited to selected obstacles or regions. For example, the magnetic field can be designed to be electromagnetic, enabling turn-on and turn-off off the magnetic fields for each individual region or obstacle at will. In other embodiments, the particles can be

released by disrupting the bond between the analyte and the capture moiety, e.g., through chemical cleavage or interruption of a noncovalent interaction. For example, some ferrous particles are linked to monoclonal antibody via a DNA linker; the use of DNAse can cleave and release the analytes from the ferrous particle. Alternatively, an antibody fragmenting protease (e.g. papain) can be used to engineer selective release. Increasing the sheer forces on the magnetic particles can also be used to release magnetic particles from magnetic regions, especially hard magnetic regions. In other embodiments, the captured analytes are not released and can be analyzed or further manipulated while retained.

FIGURE 2 illustrates the device fabrication and functionalization. The magnetized posts enable post-packaging modification of the device. This is a very significant improvement over existing art. The incompatibility of semiconductor processing parameters (high heat, or solvent sealers to bond the lid) with capture moieties (sensitive to temperature and inorganic and organic solvents) makes this device universal and compatible for functionalization with all capture moieties. Retention of the capture moieties on the obstacles (e.g., posts) by use of magnetic fields, is an added advantage over prior art that uses complex surface chemistry for immobilization. The device enables the end user to easily and rapidly charge the device with a capture moiety, or mixture of capture moieties, of choice thereby increasing the versatility of use. On-demand and 'just-in-time' one step functionalization is enabled by this device, thereby circumventing issues of on-the-shelf stability of the capture moieties if they were chemically cross-linked at production. The capture moieties that can be loaded and retained on the posts include, but not limited to, all of the cluster of differentiation (CD) receptors on mammalian cells, synthetic and recombinant ligands for cell receptors, and any other organic, inorganic molecule, or compound of interest that can be attached to any magnetic particle.

FIGURE 3 illustrates an embodiment of the device to capture and isolate cells expressing the transferrin receptor from a complex mixture. Monoclonal antibodies to CD71 receptor are readily available off-the-shelf covalently coupled to magnetic materials, such as, but not limited to ferrous doped polystyrene and ferroparticles or ferro-colloids (e.g., from Miltenyi and Dynal). The mAB to CD71 bound to magnetic particles is flowed into the device. The antibody coated particles are drawn to the posts (i.e., obstacles), floor, and walls and are retained by the strength of the magnetic field interaction between the particles and the magnetic field. The particles between the posts and those loosely retained with the sphere of influence of the local magnetic fields away from the posts, are removed by a rinse (the flow rate can be adjusted such that the hydrodynamic shear stress on the particles away from the posts is larger than the magnetic field strength).

FIGURE 4 is a preferred embodiment for application of the device to capture and release CD71+ cells from a complex mixture, e.g., blood, using holo-transferrin. Holo-transferrin is rich in iron content, commercially available, and has higher affinity constants and specificity of interaction with the CD71 receptor than its counterpart monoclonal antibody. The iron coupled to the transferrin ligand serves the dual purpose of retaining the conformation of the ligand for binding with the cell receptor, and as a molecular paramagnetic element for retaining the ligand on the posts.

In addition to the above embodiments, the device can be used for isolation and detection of blood borne pathogens, bacterial and viral loads, airborne pathogens solubilized in aqueous medium, pathogen detection in food industry, and environmental sampling for chemical and biological hazards. Additionally, the magnetic particles can be co-localized with a capture moiety and a candidate drug compound. Capture of a cell of interest can further be analyzed for the interaction of the captured cell with the immobilized drug compound. The device

can thus be used to both isolate sub-populations of cells from a complex mixture and assay their reactivity with candidate drug compounds for use in the pharmaceutical drug discovery process for high throughput and secondary cell-based screening of candidate compounds. In other embodiments, receptor-ligand interaction studies for drug discovery can be accomplished in the device by localizing the capture moiety, i.e. the receptor, on a magnetic particle, and flowing in a complex mixture of candidate ligands (or agonists or antagonists). The ligand of interest is captured, and the binding event can be detected, e.g., by secondary staining with a fluorescent probe. This embodiment enables rapid identification of the absence or presence of known ligands from complex mixtures extracted from tissues or cell digests or identification of candidate drug compounds.

Other Embodiments

All publications, patents, and patent applications mentioned in the above specification are hereby incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention.

Other embodiments are in the claims.

What is claimed is:

CLAIMS

1. A device for retaining one or more desired analytes in a sample, said device comprising a first region of magnetic obstacles disposed in a channel and a plurality of magnetic particles attached to at least one of said obstacles by a magnetic interaction.

2. The device of claim 1, wherein said channel is a microfluidic channel.

3. The device of claim 1, wherein said magnetic particles comprise a capture moiety capable of binding said one or more analytes.

4. The device of claim 3, wherein said capture moiety specifically binds a first type of analyte.

5. The device of claim 4, wherein said capture moiety comprises holo-transferrin or an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

6. The device of claim 1, further comprising a second region of magnetic obstacles, wherein a plurality of magnetic particles is attached by magnetic interaction to at least one of said obstacles in said second region.

7. The device of claim 6, wherein said obstacles in said first region are interspersed among said obstacles in said second region.

8. The device of claim 6, wherein said obstacles in said first region specifically bind a first type of analyte and said obstacles in said second region specifically bind a second type of analyte.

9. The device of claim 1, wherein at least a portion of said magnetic obstacles comprise a permanent magnet.

10. The device of claim 1, wherein at least a portion of said magnetic obstacles comprise a non-permanent magnet.

11. The device of claim 1, further comprising a magnetic force generator capable of producing a magnetic field in said magnetic obstacles.

12. The device of claim 11, wherein the magnetic field generator is capable of independently applying the magnetic field to one or more obstacles.

13. The device of claim 1, wherein said obstacles are ordered in a two-dimensional array.

14. The device of claim 1, wherein said obstacles are disposed such that said one or more analytes are capable of passing between said obstacles.

15. The device of claim 1, wherein said obstacles are disposed such that at least a portion of said one or more analytes cannot pass between said obstacles.

16. The device of claim 1, wherein said one or more analytes comprise a cell.

17. The device of claim 1, wherein said one or more analytes comprise a molecule.

18. A device for retaining one or more desired analytes in a sample, said device comprising a channel comprising a plurality of magnetic obstacles, wherein said obstacles comprise a plurality of magnetic particles, and said magnetic particles comprise a capture moiety capable of binding said one or more analytes.

19. A device for retaining one or more desired analytes in a sample, said device comprising a channel comprising a plurality of magnetic obstacles, wherein said obstacles comprise a plurality of magnetic particles, and wherein said magnetic obstacles are disposed such that at least a portion of said one or more analytes cannot pass between said obstacles.

20. The device of claim 18 or 19, wherein said channel further comprises a region of a plurality of magnetic locations and said magnetic obstacles are attached to said locations by a magnetic interaction

21. The device of claim 18 or 19, wherein said one or more analytes comprise a cell.

22. A method for retaining a first type of analyte in a sample, said method comprising the steps of:

(a) providing a sample comprising at least a first and a second type of analyte and a device comprising:

(i) a first region of magnetic obstacles disposed in a channel; and

(ii) a plurality of magnetic particles attached to at least one of said obstacles by a magnetic interaction; and

(b) introducing said sample into said device, wherein said first type of analyte is retained in said device by interaction with at least one of said obstacles.

23. A method for retaining a first type of analyte in a sample, said method comprising the steps of:

(a) providing a sample comprising at least a first and a second type of analyte and a device comprising a first region of magnetic obstacles disposed in a channel, wherein said obstacles comprise a plurality of magnetic particles; and

(b) introducing said sample into said device, wherein said first type of analyte is retained in said device by interaction with at least one of said obstacles.

24. The method of claim 22 or 23, wherein said magnetic particles are coated with a capture moiety capable of binding said first type of analyte.

25. The method of claim 22 or 23, wherein said magnetic obstacles are disposed such that at least a portion of said first type of analyte cannot pass between said obstacles.

26. The method of claim 22 or 23, wherein said first type of analyte is a particle

27. The method of claim 26, wherein said particle is a cell.

28. The method of claim 27, wherein said cell is bacterial cell, a fetal cell, or a blood cell.

29. The method of claim 22 or 23, wherein said particle is an organelle.

30. The method of claim 29, wherein said organelle is a nucleus.

31. The method of claim 22, wherein said particle is a virus.

32. The method of claim 22 or 23, wherein said first type of analyte is a molecule.

33. The method of claim 32, wherein said molecule is a nucleic acid, protein, or supramolecular complex.

34. The method of claim 22 or 23, wherein at least 60% of analytes of said first type in said sample are retained.

35. The method of claim 22 or 23, wherein at least 70% of analytes of said second type in said sample are not retained.

36. The method of claim 22 or 23, wherein said capture moiety comprises holo-transferrin or an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

37. The method of claim 24, wherein said capture moiety comprises an antibody, a protein, a peptide, or a nucleic acid.

38. The method of claim 22 or 23, wherein said device further comprises a second region of magnetic obstacles having magnetic particles attached by magnetic interaction thereto, and wherein said magnetic particles attached to said obstacles in said second region are coated with a capture moiety that selectively binds a third type of analyte.

39. The method of claim 38, wherein said obstacles in said first region are interspersed among said obstacles in said second region.

40. The method of claim 22 or 23, wherein said magnetic obstacles comprise a permanent magnet.

41. The method of claim 22 or 23, wherein said magnetic obstacles comprise a non-permanent magnet.

42. The method of claim 22 or 23, wherein said device further comprises a magnetic force generator capable of producing a magnetic field in said magnetic obstacles.

43. The method of claim 42, wherein the magnetic field generator is capable of applying the magnetic field to one or more obstacles independently.

44. The method of claim 22 or 23, wherein said obstacles are ordered in a two-dimensional array.

45. The method of claim 22 or 23, further comprising contacting a labeling moiety with said first type of analyte retained in said device

46. The method of claim 22 or 23, further comprising interrupting the magnetic interaction thereby releasing said first type of analyte from said obstacles.

47. The method of claim 22 or 23, wherein said channel is a microfluidic channel.

48. The method of claim 24, wherein said first type of analyte is specifically bound to said capture moiety.

49. The method of claim 24, wherein a candidate drug compound is attached to said magnetic particles.

50. The method of claim 49, wherein said first type of analyte is a cell, and further comprising determining the effect of said candidate drug compound on said first type of cell bound to said capture moiety.

51. The method of claim 24, wherein said capture moiety comprises a cell surface receptor.

52. The method of claim 51, wherein said sample comprises candidate ligands for said cell surface receptor.

53. The method of claim 24, wherein said device further comprises a second plurality of magnetic particles attached to at least one of said obstacles by a

magnetic interaction, wherein said magnetic particles are coated with a second capture moiety capable of binding said first type of analyte.

54. The method of claim 53, wherein said plurality of particles in step (a)(ii) and said second plurality of particles are disposed on the same obstacle.

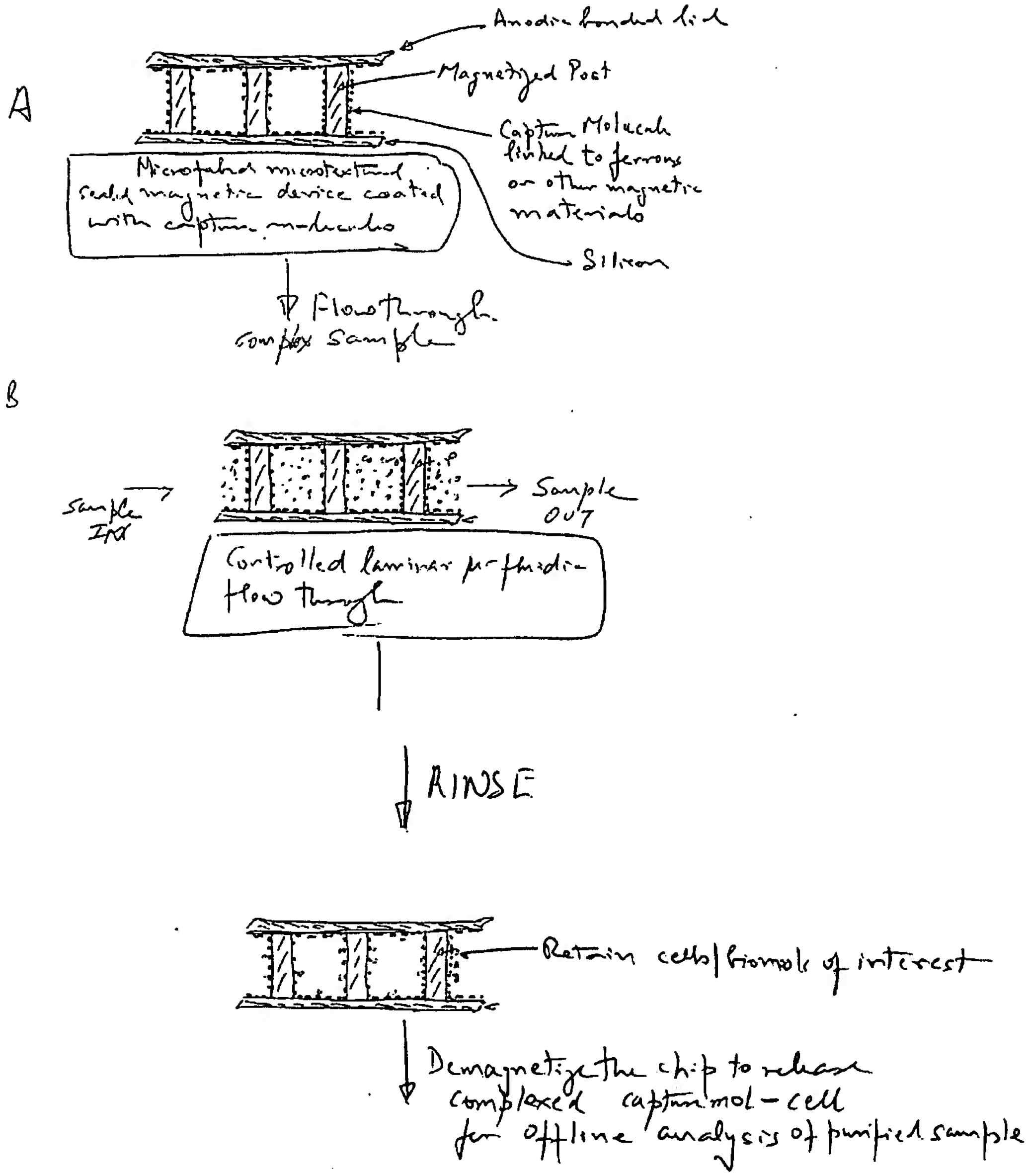
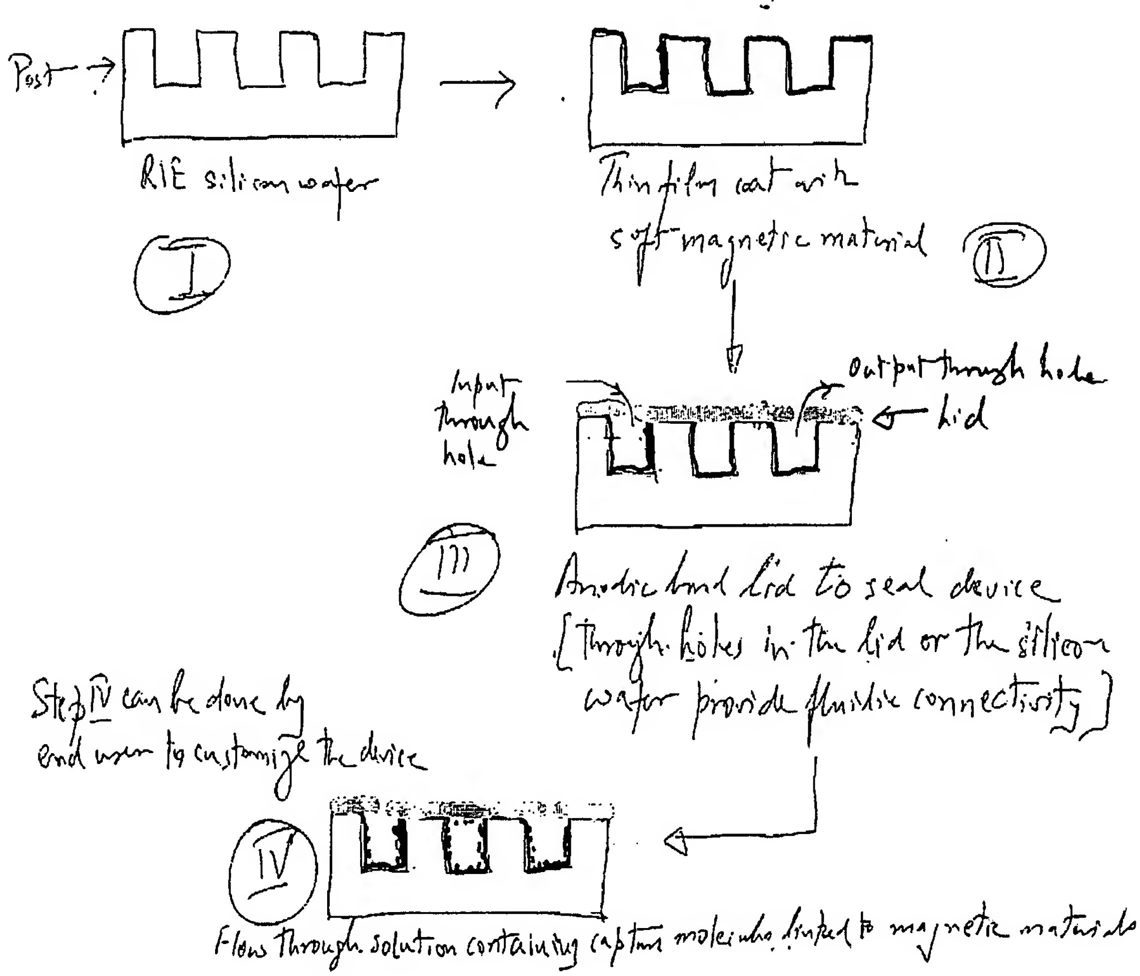


Fig 1: Cross-sectional view of device (A) & process flow for cell isolation & release for analysis (B & C)

Fig 2. Device fabrication



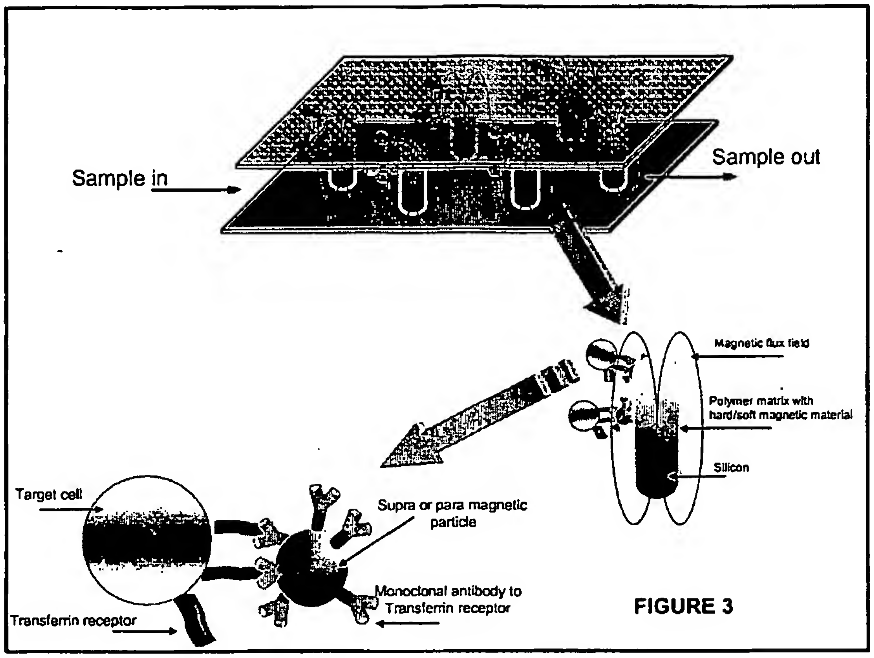


FIGURE 3

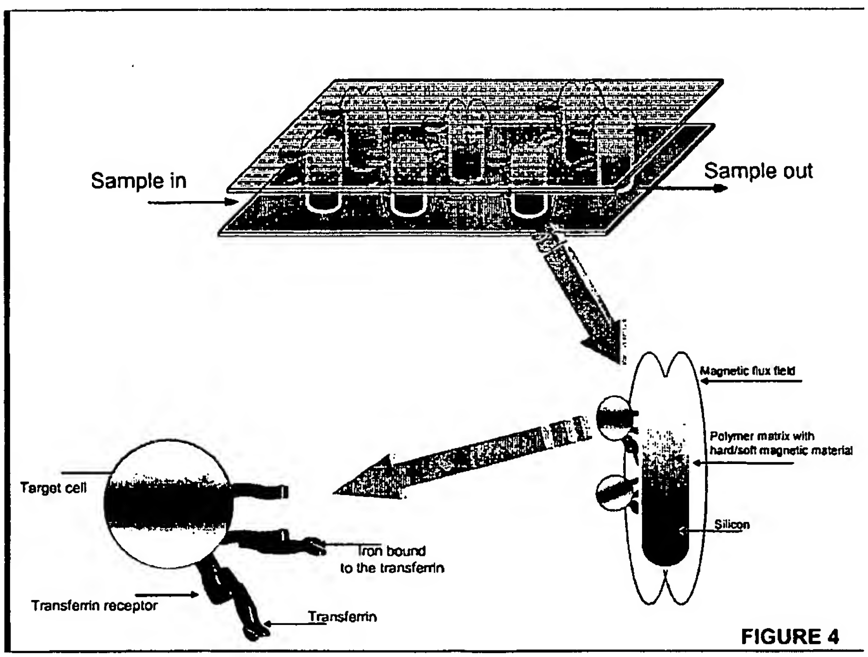


FIGURE 4